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Simultaneous determination of imipramine and amitryptiline by derivative spectrophotometry

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Abstract: Three methods are proposed for the simultaneous determination of imipramine and amitryptiline by derivative spectrophotometry, one of them using both first- and second-derivative spectra, and the others using the first- and second-derivative spectra, respectively, obtained from a "diode array" spectrophotometer. The methods allow the determination of 0.62–10.14 μg ml^{-1} of imipramine, and 0.63–10.04 μg ml^{-1} of amitryptiline, and have been applied to their determination in blood serum.

Keywords: *Imipramine; amitryptiline; tricyclic antidepressants; derivative spectrophotometry; simultaneous determination.*

Introduction

Imipramine and amitryptiline are the most widely employed of the tricyclic antidepressants, since their introduction by Khun in 1958 [1], and many methods have been proposed for their determination, namely, spectrophotometric and spectrofluorimetric [2–5], potentiometric [6], polarographic [7], IES [8], EMIT [9] and, in particular, HPLC [10–11], despite requiring specialized apparatus and the technique being laborious as a routine analysis. However, few methods have been directed towards their simultaneous determination in mixtures [12].

Three methods for the simultaneous determination of imipramine and amitryptiline, based on derivative spectrophotometry, are developed and compared in this work.

This technique is being used more and more frequently in drug analysis due, fundamentally, to its advantages in the elimination of matrix effects, improved resolutions of spectra and the possibility of carrying out the qualitative and quantitative analysis of multicomponent systems [13].

Experimental

Apparatus

A Perkin–Elmer 550S UV–vis spectrophotometer equipped with 1-cm path length quartz cells, and a Hewlett Packard diode array HP8452A spectrophotometer with a 1-cm path length quartz cell, coupled to a Hewlett Packard Vectra ES Computer and Hewlett Packard Think Jet printer were used.

Reagents

Standard aqueous solutions of imipramine and amitryptiline hydrochlorides were prepared by weighing from commercial products (Sigma). These solutions were maintained at room temperature, in the dark. Acetic acid–sodium acetate buffer solution (pH 5.10). The ionic strength was maintained constant at 0.25 with $NaClO_4$.

All the reagents used were of analytical reagent grade and the water was deionized.

Simultaneous determination of imipramine and amitryptiline

To 25-ml calibrated flasks were added 2.5 ml of 2.5 M $NaClO_4$ solution, 3.0 ml of buffer solution (pH 5.10), 5.0 ml of ethanol, and imipramine and amitryptiline in the range 15.4–253.5 and 15.7–251.1 μg, respectively. The solutions were made up to volume with deionized water and the first and second-derivative spectra were then recorded against a reagent blank, prepared in a similar manner without antidepressant, between 190–350 nm in both spectrophotometers. The conditions used in the 550S spectrophotometer were: scan speed, 120 nm min^{-1}; chart speed, 60 nm min^{-1}; and response time, 7 s. The maximum

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Table 1
Statistical analysis of methods for the determination of imipramine and amitryptiline

Equation	r	SD	RSD	DL
	Method 1			
$D_1 = -1.86 \times 10^{-1} + 4.32 \times 10^{-1} C_I$	0.996	1.17×10^{-1}	5.21	0.777
$I_2 = -1.22 \times 10^{-1} + 4.58 \times 10^{-1} C_I$	0.997	1.12×10^{-1}	4.79	0.736
$A_2 = -6.16 \times 10^{-3} + 1.30 C_A$	0.999	1.94×10^{-1}	2.79	0.447
	Method 2			
$H_1 = 3.23 \times 10^{-5} + 4.00 \times 10^{-4} C_I$	0.999	4.30×10^{-5}	1.85	0.303
$H_2 = -4.86 \times 10^{-4} + 2.04 \times 10^{-3} C_A$	0.996	5.84×10^{-4}	5.72	0.845
	Method 3			
$H_3 = -5.92 \times 10^{-6} + 7.52 \times 10^{-5} C_I$	0.999	8.30×10^{-6}	2.08	0.714
$H_4 = 1.41 \times 10^{-6} + 8.15 \times 10^{-5} C_A$	0.999	1.02×10^{-5}	2.33	0.374
$H_5 = 2.76 \times 10^{-6} + 6.66 \times 10^{-6} C_A$	0.943	7.19×10^{-6}	8.80	3.244

C = μg ml^{-1}; SD = standard deviation; RSD = relative standard deviation; DL = detection limit (μg ml^{-1}).

and minimum ordinates were ±0.05 and ±0.005 for the first and second derivatives, respectively. In the Hewlett Packard spectrophotometer, the integration time used was 1 s.

In the first- and second-derivative spectra recorded in the 550S spectrophotometer the signals comprised in the range 295 and 315 nm and 240 and 265 nm were measured, respectively (method 1).

For methods 2 and 3, the height of the peaks in the first-order derivative spectrum were measured at 300 and 252 nm and those of the second-order derivative at 268 and 254 nm, respectively.

The comparison of the signals measured with the calibration curves (Table 1) allows the determination of both antidepressants.

Simultaneous determination of imipramine and amitryptiline in blood serum

Two millilitres of serum, containing between 140–420 μg of both antidepressants, were made alkaline (pH 11.5) with 0.1 M NaOH; 50.0 ml of *n*-hexane were added and the mixture shaken and centrifuged for about 10 min. The aqueous layer was discarded and 7.0 ml of 6 M HCl were added to the organic layer. Then, this mixture was shaken and centrifuged for about 10 min. 5.0 ml of the acidic extract were neutralized to pH 3.5 and analysed, as described above, against a blank treated in the same way.

Results and Discussion

Figure 1 shows the zero-order absorption spectra, first- and second-order derivative spectra, for solutions of imipramine, amitryptiline and the mixture of both at pH 5.10, ethanol–water 20% (v/v) and I = 0.25 M. No changes were observed in these spectra in the pH range 1–11 and the presence of ethanol was necessary for the spectral signals of the mixture to be additive (percentages of ethanol above 20% do not improve the resolution). It can be observed in the above mentioned figure that imipramine and amitryptiline, with absorption maxima at 245 and 215 nm for the former and 218 nm with a shoulder at 235 nm for the latter, present strongly overlapping zero-order spectra that do not allow their simultaneous determination.

In the first-derivative spectrum of the mixture [Fig. 1(b)] the distance between the shoulders at 295 and 315 nm (D_1) corresponds to the amount of imipramine present; while in the second-derivative spectrum [Fig. 1(c)] the distance between the maximum at 240 nm and the minimum at 265 nm (D_2) is the sum of those corresponding to imipramine (I_2) and amitryptiline (A_2). In order to carry out the determination of both antidepressants, calibration curves were constructed for imipramine (D_1 vs C_1 and I_2 vs C_1) and amitryptiline (A_2 vs C_A); the amount of imipramine present is determined from the measurement D_1 and hence the corresponding distance I_2; if I_2 is subtracted from the distance D_2, A_2 is obtained, allowing calculation of the amount of amitryptiline in the mixture. The equations of the calibration straight lines, as well as their standard deviations and relative standard deviations [14, 15] are given in Table 1 (Method 1).

In view of the laboriousness of the proposed method, that requires recording of the first-

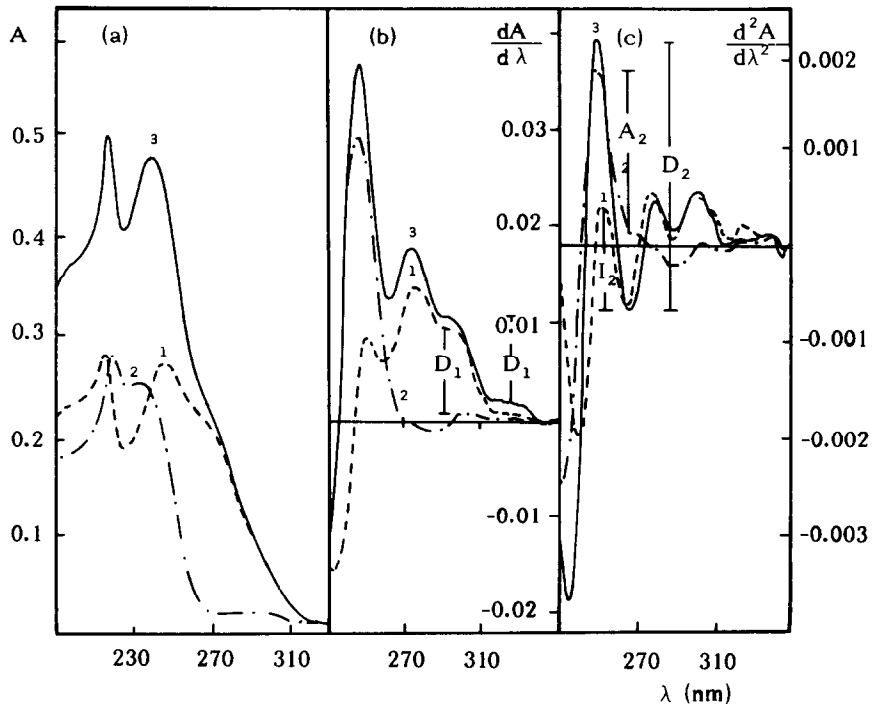

Figure 1
(a) Absorption, (b) first- and (c) second-derivative spectra of imipramine and amitryptiline, pH 5.10. (1) C_I =5.387 μg ml^{-1}; (2) C_A = 5.336 μg ml^{-1}; (3) C_I = 5.387 μg ml^{-1} and C_A = 5.336 μg ml^{-1}. For the meaning of D_1, D_2, I_2 and A_2 see text.

and second-derivative of each of the samples, and the manual measuring of the distances in each of them, it was considered worthwhile to find a method that would facilitate the resolution of the mixture by using a single spectrum and wherein the distances could be measured with the aid of a computer. The first- and second-derivative spectra were thus studied separately, using a diode array spectrophotometer, which on achieving a greater reproducibility, by setting the wavelengths, allows exact measurements to be used rather than peak-to-peak distances only. Both antidepressants can be determined simultaneously by applying the "zero-crossing" method [16, 17] to each of the derivative spectra.

Figure 2(a) shows a series of first-derivative spectra of mixtures containing 5.34 μg ml^{-1} of amitryptiline and amounts of imipramine within the range 0.63–10.14 μg ml^{-1}. An isosbestic point at 252 nm, the "zero-crossing" wavelength of imipramine, can be observed herein, as well as the fact that the height at 300 nm (H_1) is proportional to the concentration of imipramine in the sample. Likewise, Fig. 2(b) presents the first-derivative spectra of solutions containing 5.39 μg ml^{-1} of imipramine and amounts of amitryptiline varying between 0.63–10.04 μg ml^{-1}; an isosbestic point at 240 nm, "zero-crossing" of amitryptiline, is observed, and the height at 252 nm (H_2) is proportional to the amount of amitryptiline in the sample. The equations of the calibration straight lines H_1 vs C_I and H_2 vs C_A are given in Table 1, together with the statistical data of interest (method 2).

A similar study of the second-derivative spectra was carried out. Thus, the spectrum of imipramine crosses through zero at 268, 259 and 243 nm, as does that of amitryptiline at 253 and 237 nm. Since the diode array spectrophotometer only permits the selection of eves wavelengths, 268 nm was chosen for the determination of amitryptiline and 254 nm for imipramine, with the corresponding correction for the predetermined amount of amitryptiline present.

In Figs 3(a) and 3(b) it can be observed that the heights of the maxima at 254 nm (H_3) and 268 nm (H_4) are proportional to the concentrations of imipramine and amitryptiline, respectively. The calibration straight lines H_3 vs C_I, H_4 vs C_A and H_5 vs C_A (correction at 254 nm for imipramine in mixtures) and their most significative characteristics are shown in Table 1 (method 3).

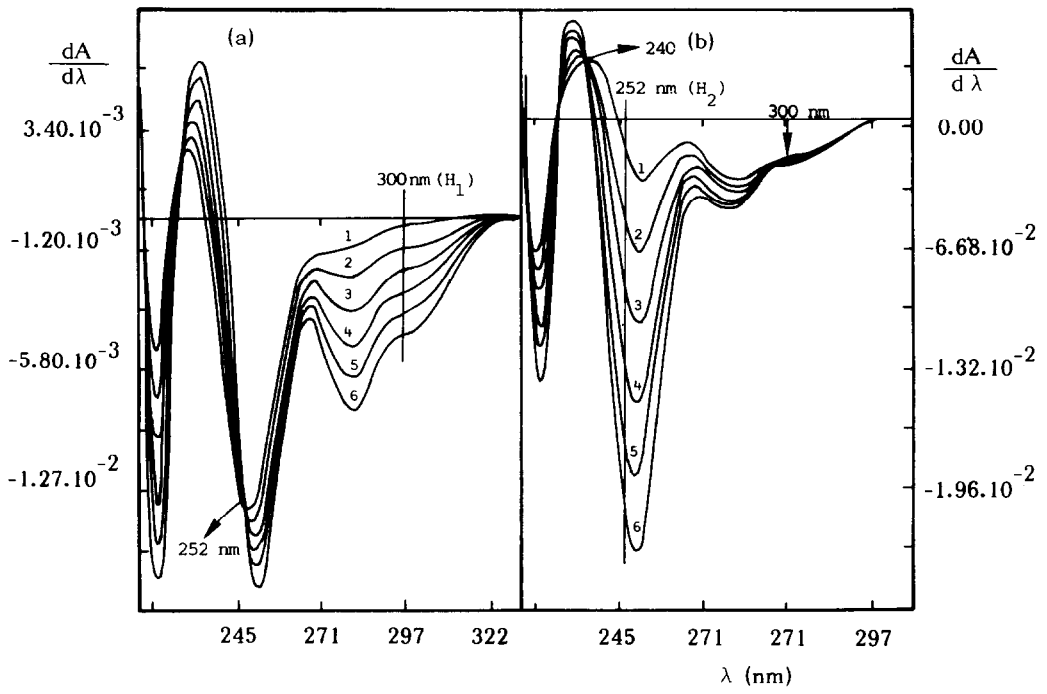

Figure 2
First-derivative spectra, pH 5.10, of (a) C_A = 5.336 μg ml^{-1} = cte, C_I = (1) 0.634 μg ml^{-1}; (2) 2.535 μg ml^{-1}; (3) 4.437 μg ml^{-1}; (4) 6.338 μg ml^{-1}; (5) 8.239 μg ml^{-1}; (6) 10.141 μg ml^{-1}; (b) C_I = 5.387 μg ml^{-1} = cte, C_A = (1) 0.628 μg ml^{-1}; (2) 2.511 μg ml^{-1}; (3) 4.395 μg ml^{-1}; (4) 6.278 μg ml^{-1}; (5) 8.161 μg ml^{-1}; (6) 10.045 μg ml^{-1}.

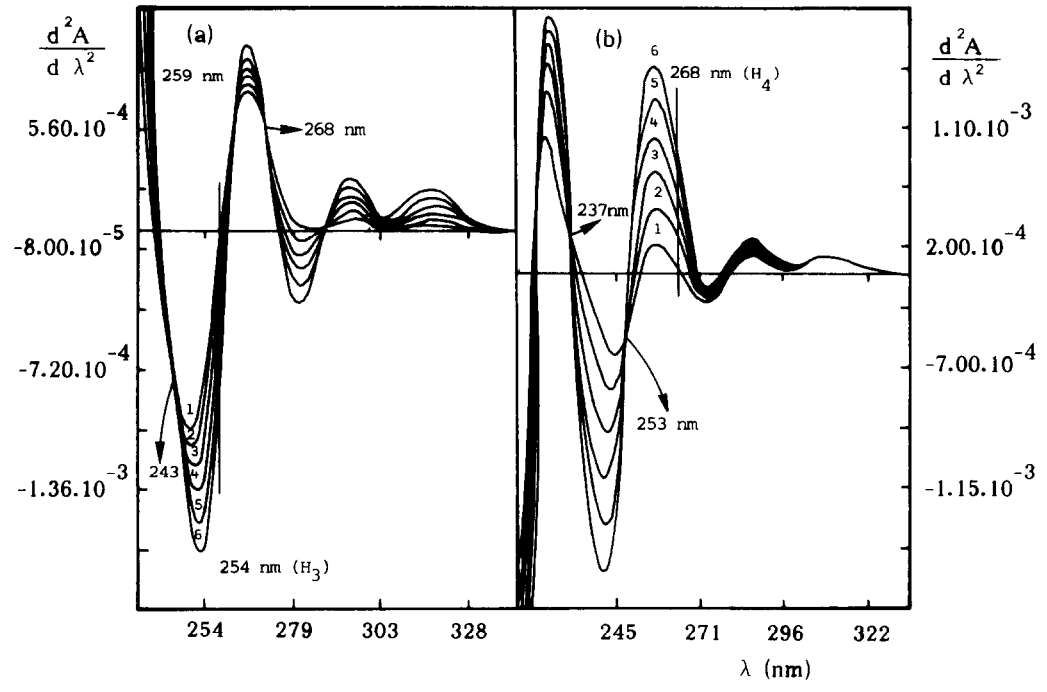

Figure 3
Second-derivative spectra, pH 5.10, of (a) C_A = 5.336 μg ml^{-1} = cte, C_I = (1) 0.634 μg ml^{-1}; (2) 2.535 μg ml^{-1}; (3) 4.437 μg ml^{-1}; (4) 6.338 μg ml^{-1}; (5) 8.239 μg ml^{-1}; (6) 10.141 μg ml^{-1}; (b) c_I = 5.387 μg ml^{-1} = cte, C_A = (1) 0.628 μg ml^{-1}; (2) 2.511 μg ml^{-1}; (3) 4.395 μg ml^{-1}; (4) 6.278 μg ml^{-1}; (5) 8.161 μg ml^{-1}) (6) 10.045 μg ml^{-1}.

Table 2
Statistical parameters for the determination of imipramine and amitryptiline in mixtures

Statistic	Method 1		Method 2		Method 3	
	I	A	I	A	I	A
	(1) 8.239 μg ml^{-1} of imipramine, 2.511 μg ml^{-1} of amitryptiline					
x	8.169	2.398	8.166	2.665	8.191	2.544
SD	3.03×10^{-1}	1.46×10^{-1}	1.43×10^{-1}	8.07×10^{-2}	8.47×10^{-2}	7.93×10^{-2}
RSD (%)	3.71	6.11	1.75	3.02	1.03	3.12
t_{exp}	0.231	0.774	0.510	1.908	0.560	0.416
t_{exp}	0.980	3.284	2.163	8.095	2.400	1.765
	(2) 5.387 μg ml^{-1} of imipramine, 5.336 μg ml^{-1} of amitryptiline					
x	5.319	5.297	5.443	5.484	5.437	5.414
SD	2.04×10^{-1}	2.10×10^{-1}	6.10×10^{-2}	1.98×10^{-1}	1.42×10^{-1}	1.75×10^{-1}
RSD (%)	3.84	3.97	1.12	3.61	2.61	3.24
t_{exp}	0.333	0.186	0.918	0.747	0.352	0.446
t_{exp}	1.413	0.789	3.895	3.169	1.493	1.892
	(3) 2.535 μg ml^{-1} of imipramine, 8.161 μg ml^{-1} of amitryptiline					
x	2.530	8.096	2.695	8.116	2.575	8.245
SD	1.67×10^{-1}	1.75×10^{-1}	1.45×10^{-1}	1.81×10^{-1}	1.40×10^{-1}	2.62×10^{-1}
RSD (%)	6.61	2.16	5.39	2.23	5.45	3.18
t_{exp}	0.017	0.371	1.103	0.248	0.286	0.322
t_{exp}	0.073	1.576	4.681	1.055	1.212	1.365

Statistical study

The precision and accuracy of the three methods were assessed with three different ratios of the antidepressants within the range of concentration in which the method can be applied: (1) 8.24 μg ml^{-1} of imipramine and 2.51 μg ml^{-1} of amitryptiline; (2) 5.39 μg ml^{-1} of imipramine and 5.34 μg ml^{-1} of amitryptiline; and (3) 2.53 μg ml^{-1} and 8.16 μg ml^{-1} of imipramine and amitryptiline, respectively. Three series of six samples each were thus prepared for each ratio under study, the results found being given in Table 2, where the good results obtained by the three methods can be observed both for the determination of imipramine and that of amitryptiline, although method 2 leads in same cases to results affected by systematic error.

The applicability of the proposed methods in the resolution of binary mixtures of imipramine and amitryptiline was checked by assaying synthetic mixtures of these compounds in different proportions. The results obtained are given in Table 3 and show that the simultaneous determination of imipramine and amitryptiline can be achieved by following these methods, the best results being afforded by method 3.

Effect of foreign ions

The effect of several interfering ions on the determination of 5.39 μg ml^{-1} of imipramine and 5.34 μg ml^{-1} of amitryptiline was investigated by applying the described procedures to solutions containing a 100-fold (m/m) ratio of interfering ion or substance to the species to be determined. If interference occurred, this ratio was reduced until the interference ceased. The criterion for interference was a deviation of more than ±5% from the concentration of imipramine and amitryptiline taken. The results are shown in Table 4.

Table 3
Simultaneous determination of imipramine and amitryptiline in synthetic mixtures

I_{added}	A_{added}	I_{found}	% E	A_{found}	% E
		Method 1			
2.535	2.511	2.523	−0.47	2.865	14.10
5.070	5.022	4.679	−7.71	5.316	5.85
7.606	7.534	7.265	−4.48	7.956	5.60
7.606	5.022	7.157	5.90	5.392	7.37
7.606	10.045	7.911	4.01	10.143	0.97
7.606	2.511	7.049	7.32	2.752	9.59
10.141	7.534	10.390	2.45	7.654	1.59
5.070	7.534	4.463	11.97	8.144	8.09
10.141	5.022	10.390	2.45	5.052	0.59
2.535	10.045	2.416	4.69	10.331	2.85
2.535	5.022	2.524	0.43	5.203	3.60
4.437	3.767	4.248	4.26	3.921	1.43
		Method 2			
2.535	2.511	2.675	5.52	2.779	10.67
5.070	5.022	5.400	5.40	5.358	6.69
7.606	7.534	7.978	4.89	7.853	4.23
7.606	5.022	7.855	3.27	5.309	5.71
7.606	10.045	7.978	4.89	10.397	3.50
7.606	2.511	7.904	3.91	2.887	14.97
10.141	7.534	10.531	3.84	7.902	4.88
5.070	7.534	5.425	7.00	7.902	4.88
10.141	5.022	10.531	3.84	5.407	7.66
2.535	10.045	3.018	19.05	10.495	4.48
2.535	5.022	2.797	10.33	5.358	6.69
4.437	3.767	4.614	4.00	4.081	8.33
		Method 3			
2.535	2.511	2.702	6.58	2.608	3.86
5.070	5.022	5.189	2.34	5.217	3.88
7.606	7.534	7.771	2.17	7.717	2.43
7.606	5.022	7.840	3.07	5.205	3.64
7.606	10.045	7.584	0.29	10.217	1.71
7.606	2.511	7.772	2.18	2.762	10.11
10.141	7.534	10.203	0.61	7.741	2.74
5.070	7.534	5.124	1.06	7.766	3.08
10.141	5.022	10.356	2.12	5.265	4.84
2.535	10.045	2.604	2.72	10.338	2.92
2.535	5.022	2.655	4.73	5.217	3.88
4.437	3.767	4.566	2.90	3.912	3.85

Simultaneous determination of imipramine and amitryptiline in blood serum

The proposed methods were applied to the simultaneous determination of imipramine and amitryptiline in blood serum, in accordance

Table 4
Interference levels of foreign ions in the simultaneous determination of imipramine and amitryptiline. Concentrations: imipramine, 5.387 μg ml^{-1}; amitryptiline, 5.336 μg ml^{-1}

[Ion]/[I]	Ion added
100:1	Cl^-, F^-, Ca^{2+}, Mg^{2+}, K^+, Li^+ starch, glucose, urea
5:1	Thiourea
1:1	Fe^{3+}*
≤1	Fe^{3+}, uric acid, ascorbic acid, $S_2O_3^{2-}$

*In excess of F^-.

Table 5
Simultaneous determination of imipramine and amitryptiline in blood serum

A_{added}	A_{found}	% E	I_{added}	I_{found}	% E
		Method 1			
100.00	102.55	2.55	200.00	199.52	0.24
200.00	190.68	4.66	100.00	102.55	2.55
300.00	284.75	5.08	100.00	100.80	0.01
200.00	207.20	3.60	200.00	200.90	0.45
		Method 2			
100.00	95.99	4.01	200.00	176.59	11.70
200.00	198.54	0.73	100.00	104.51	4.51
300.00	285.41	4.86	100.00	95.20	4.80
200.00	204.14	2.07	200.00	207.20	3.60
		Method 3			
100.00	75.68	24.32	200.00	216.23	8.11
200.00	224.42	12.21	100.00	93.05	6.95
300.00	283.40	5.53	100.00	102.90	2.90
200.00	196.57	1.71	200.00	184.80	7.60

μg Added and found of imipramine and amitryptiline.

with the procedure described in the Experimental section, the results obtained being summarized in Table 5.

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